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The Importance of Rhizosphere Interactions in the Biological Control of Plant Parasitic Nematodes—a Case Study using *Verticillium chlamydosporium**

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Abstract: *Verticillium chlamydosporium* has provided considerable control of root knot nematodes in a range of laboratory experiments and is one of the most promising natural enemies with potential as a biological control agent so far tested. From a detailed study of the biology and ecology of the fungus, it has been possible to identify the key factors which affect its efficacy and a strategy for its exploitation has been developed. The fungus survives in soil throughout a growing season and its concentration in soil may be increased by repeated applications. The rapid multiplication of root-knot nematodes on susceptible crops means that control must be extremely effective to prevent crop damage. If suitable methods of production and application can be developed, *V. chlamydosporium*, in conjunction with other methods such as the rotation of poor hosts, may provide adequate control, but the reliability of such approaches needs extensive testing. Although genetic manipulation offers the possibility of enhancing the ability of the fungus to kill nematodes, such an approach will require much research and, in the short term, the development of the fungus will rely on the exploitation of carefully selected wild types.

Key words: root knot nematodes, *Verticillium chlamydosporium*, biological control, rhizosphere interactions.

1 INTRODUCTION

Some bacteria and fungi in soil have been demonstrated to be effective control agents for specific nematode pests. Thus, the bacterium *Pasteuria penetrans* (Thorne) Sayre & Starr has effectively controlled a population of *Meloidogyne arenaria* (Neal) Chitwood on peanuts in Florida.[1] Also, for the past 20 years the cereal cyst nematode *Heterodera avenae* Wollenweber has been prevented from increasing in monocultures of susceptible cereal crops because of nematophagous fungi which parasitise nematode females and eggs. The decline of cereal cyst nematodes was first reported by Gair *et al.*[2] and the role of fungal parasites in the decline phenomenon was elucidated by Kerry *et al.*[3] The natural control of the cereal cyst nematode provides a demonstration of sustainable nematode management in intensive agriculture and, despite the widespread occurrence of this pest in the UK and elsewhere in Northern Europe, it causes relatively little damage in many soils.

The importance of natural control has been demonstrated through the application of treatments which enhance or suppress the activity of natural enemies in soil and the influence on nematode infestations measured. Formalin (38% formaldehyde) is in itself a poor nematicide, and, applied as a soil drench, effectively killed the nematophagous fungi responsible for control of cereal cyst nematodes and resulted in large increases in the pest population (Fig. 1). In soils suppressive to nematode pests, only one or two natural enemies have

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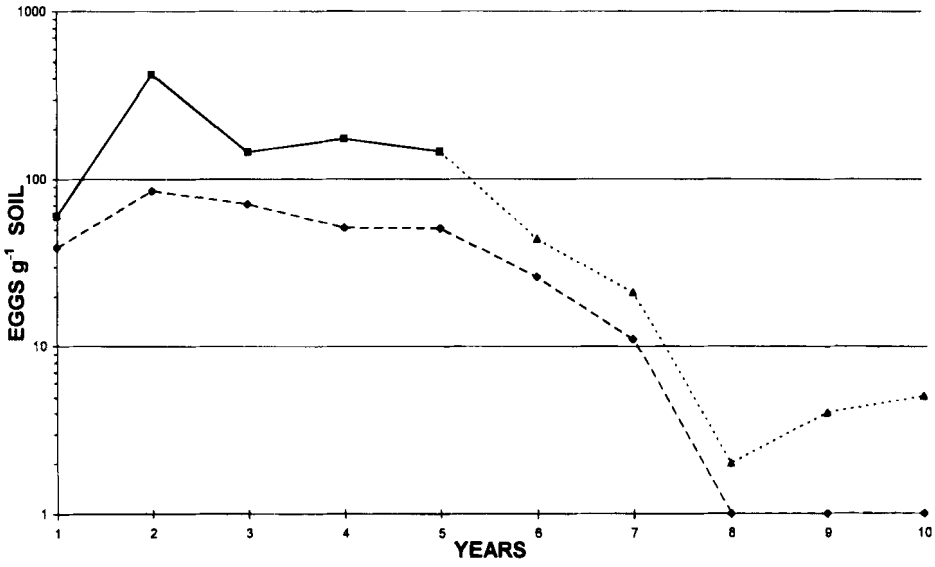

Fig. 1. Changes in the pre-cropping population densities (eggs g^{-1} soil) of the cereal cyst nematode, *Heterodera avenue*, in monocultures of susceptible spring barley *cv.* Triumph in untreated soil and soil treated with formalin (38% formaldehyde) each year for the first five years of cropping. (■) formalin treated soil; (▲) residual formalin treatment; (◆) untreated soil. (Means of four replicates).

been considered responsible for the natural control observed.[4] However, it has not been possible to introduce single species of microbial agents and provide levels of control similar to those that occur naturally. Several agents have been produced commercially but their success has been limited and there is a need for sound biological and ecological information in order to assess rationally the potential of selected organisms. This paper reviews recent progress in the development of *Verticillium chlamydosporium* (Goddard) as a biological control agent for root-knot nematodes and highlights the key role of the host plant in determining the efficacy of the fungus.

2 TRITROPHIC INTERACTIONS IN THE RHIZOSPHERE

Interactions in the rhizosphere (Fig. 2) have a major effect on the numbers of nematodes parasitised by such natural enemies as *V. chlamydosporium* but these have been little studied in the development of biological control strategies for plant parasitic nematodes. Applied nematologists have done much to increase knowledge of the relationship between the numbers of a pest species and crop damage and, for several nematodes, mathematical models have been produced which enable predictions of yield losses to be made.[5,6] The susceptibility of the host plant will influence the number of nematodes attacking the roots, the rate of development of the nematodes and their multiplication, all factors which are likely to influence the impact of the natural enemy on the population dynamics of the nematode. Although a wide range of organisms has been reported to attack nematodes,[7] their influence on nematode populations is only poorly understood. Entomologists have long recognised the need for research on the population dynamics of pests and natural enemies in the development of biological control programmes,[8] and have acknowledged the importance of the density dependence of natural enemies in these interactions. Few nematologists have studied the role of natural enemies in nematode population dynamics and research has concentrated on surveys for potential new agents, empirical tests in which single agents are added to soil as inundative treatments for nematode control and basic studies on infection processes and nutrition of selected organisms. However, pioneering work by Jaffee[9] has demonstrated the importance of density dependence in the biological control of nematodes. Part of the problem in understanding the quantitative relationships between nematodes and their microbial pathogens and parasites is due to the great difficulties in estimating the density of these natural enemies in soil. Although the numbers of some nematophagous bacteria

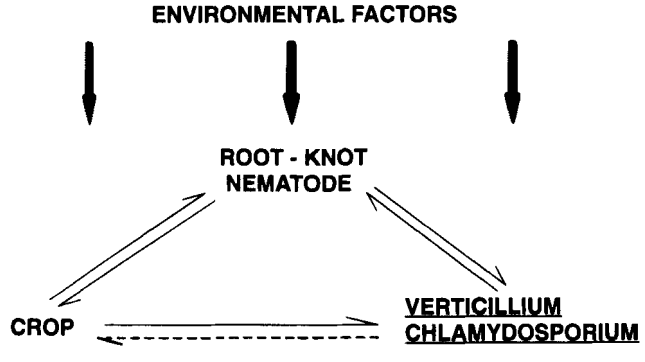

Fig. 2. The key components of nematode, crop and natural enemy that form the basis of tritrophic interactions in the rhizosphere.

can be estimated in bioassays,[10] the sizes of populations of nematophagous fungi which have been most studied as potential biological control agents for nematodes are notoriously difficult to estimate. Several methods have been used, including estimates of numbers of nematodes parasitised, physical extraction of spores,[11] most probable number techniques[12] and selective media.[13–15] All these approaches have major limitations. The use of dilution plate techniques in conjunction with a selective medium has greatly increased understanding of the survival and proliferation of *V. chlamydosporium* in soil.[15] However, such methods do not allow the separation of propagules (hyphae, conidia and chlamydospores) which occur together in soil, so that apparent changes in abundance may result from increased vegetative growth or from sporulation.[16] Hence, the fungus may be in very different physiological states and still give rise to similar estimates of abundance based on the numbers of colonies developing on dilution plates. There is a need for alternative methods of estimating fungal populations in soil and the use of monoclonal antibodies and ELISA techniques is currently being assessed in this laboratory.

Verticillium chlamydosporium does not have a direct effect on root growth. In studies on a range of crops, no damage to roots has been observed and the fungus appears to be confined to the rhizosphere.[17] However, the plant itself has major effects on the growth of the fungus on roots and this has a profound influence on the level of nematode control achieved. An understanding of the relationship between the host plant and *V. chlamydosporium* is crucial to the use of this fungus as a biological control agent and this is discussed in detail below.

3 *VERTICILLIUM CHLAMYDOSPORIUM* AS A FACULTATIVE PARASITE

Verticillium chlamydosporium does not depend solely on nematodes for its nutrition; it is able to colonise other soil organisms including fungi and the eggs of snails[18] and the rhizospheres of healthy plants, but colonisation of soil organic matter is limited.[18,19] The fungus proliferates in the rhizosphere and colonises the egg masses of root-knot nematodes (Fig. 3) or female cyst nematodes as they enlarge and rupture the root cortex and are exposed on the root surface. The causes for the switch of the fungus from saprophyte in the rhizosphere to nematode parasite are not understood. It does not produce specialised structures to capture and infect nematodes and it is only capable of parasitising nematode eggs or the sedentary females of certain nematode groups such as cyst and root-knot nematodes. Infection of eggs results from contact between the vegetative mycelium and the eggshell, on which develops an appressorium produced terminally or laterally on a

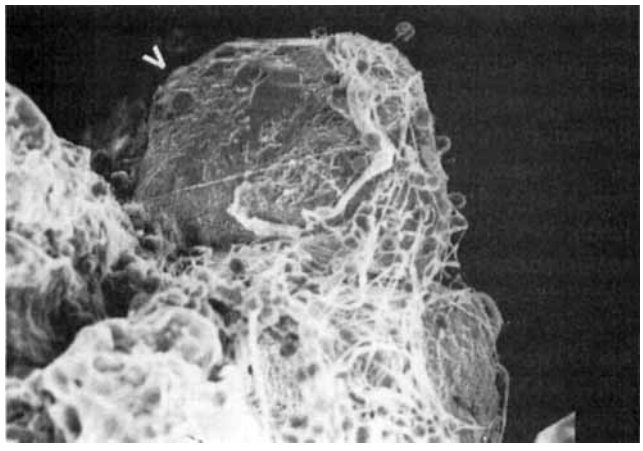

Fig. 3. A scanning electron micrograph illustrating hyphae and chlamydospores of *Verticillium chlamydosporium* on the surface of a tomato plant root and colonisation of the nematode egg mass (arrowed).

hypha (Segers R., 1995, pers. comm.). The appressorium gives rise to a penetration peg which grows through the eggshell and produces a post-infection bulb from which hyphae develop and destroy the egg contents. All stages of embryonic development are attacked by the fungus, but immature eggs are more susceptible than those containing second-stage juveniles. During the infection process, *V. chlamydosporium* produces a subtilisin which breaks down the outer membrane of the eggshell and exposes the chitin layer of root-knot nematode eggs.[20] The amounts of this enzyme produced *in vitro* by different isolates of the fungus differ considerably and the subtilisin is not active against eggs of *Globodera rostochiensis* (Wollenweber), which are colonised only slowly (Segers R., 1995, pers. comm.); hence, this enzyme may be a virulence- and specificity-determining factor. Similar enzymes are produced by the nematophagous fungi *Paecilomyces lilacinus* (Thom) Samson,[21] *Verticillium suchlasporium* Gams & Dackman[22] and *Arthrobotrys oligospora* Fres.[23] and by the entomopathogenic fungi *Metarhizium anisopliae* (Metsch.) Sorokin and *Verticillium lecanii* (Zimm.) Viégas, but not by the plant pathogens *Verticillium albo-atrum* Reinke & Berth. and *Verticillium dahliae* Kleb.[20]

All stages of *V. chlamydosporium* occur in soil (Fig. 4). Chlamydospores enable the fungus to survive when nematode hosts are scarce[24] and may be used to establish the fungus in soil.[17] Spread in soil due to hyphal growth is very limited[19] although more extensive growth occurs in the rhizosphere. In laboratory tests, the fungus spread approximately 50 cm down the soil profile in nine weeks, but only a small proportion (1%) of the inoculum added to soil achieved this and most inoculum spread less than 15 cm.[25] Spread was considered to be mainly the result of conidia being transported by water movement and is likely to be much less extensive in undisturbed soils. Although soil invertebrates are considered important agents for the

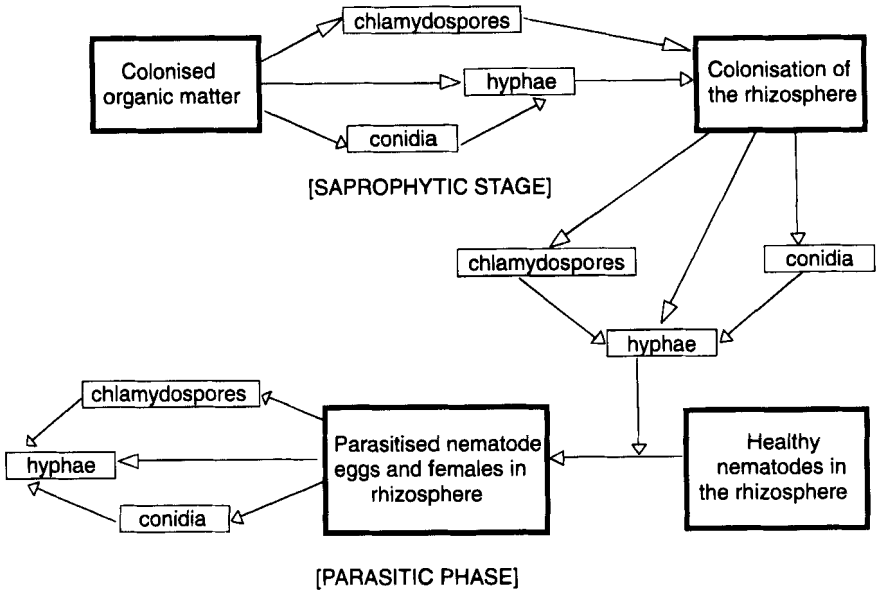

Fig. 4. Stages in the saprophytic and parasitic development of *Verticillium chlamydosporium* in soil.

dispersal of microorganisms in soil, nothing is known of their significance in the dispersal of *V. chlamydosporium*. The slow build-up of nematophagous fungi in nematode-suppressive soils indicates that their dispersal is limited.

4 THE ROLE OF THE HOST PLANT IN THE INTERACTIONS BETWEEN *VERTICILLIUM CHLAMYDOSPORIUM* AND ROOT-KNOT NEMATODES

Although many isolates of *V. chlamydosporium* are capable of proliferating and surviving in soil, only those capable of colonising the rhizosphere are able to parasitise sufficient nematode eggs to limit the multiplication of root-knot populations.[17] Isolates of the fungus differ in their ability to colonise the rhizosphere[16] and careful selection is necessary to identify the most effective colonisers. Also, plant species differ markedly in their ability to support *V. chlamydosporium* in their rhizospheres. In a pot test, 17 plant species were grown in non-sterilised soil inoculated with chlamydospores (5000 g^{-1} soil) at the time of planting. After seven weeks, the abundance of the fungus in the rhizosphere was estimated using methods described by Bourne *et al.*,[16] each plant species was replicated five times with one replicate in each block on the glasshouse bench. In each block, plants were ranked in order of the amount of fungus detected in the rhizosphere; selected data for poor and good hosts for the fungus are presented in Table 1. Kale supports most and tomato plants least growth in all blocks but there were marked differences between replicates of the same species; positions of plants relative to the glasshouse lights and/or differences in watering may have affected growth of the fungus in the rhizosphere by influencing root exudates. Hence, in experiments to test the efficacy of the fungus as a biological control agent, nematodes may be exposed to very different densities of the fungus in different blocks and this may result in considerable variation in the control achieved with different replicates of the same treatment.

In general, the presence of the nematode substantially increases the density of the fungus on infected roots[18] and this increase is much greater on plants which support little growth on healthy roots. Hence, nematode-infected tomato roots supported more growth of *V. chlamydosporium* than the rhizospheres of infected kale. More nematodes invaded the roots of tomato plants compared to kale and this either affected

TABLE 1
Effects on the Growth of *V. chlamydosporium* in the Rhizosphere of Some Crops[a]

	Colonisation rating				
	Block				
Plant host	*A*	*B*	*C*	*D*	*E*
Kale	1	1	1	1	1
Cabbage	3	2	2	4	4
Cowpea	12	12	14	12	6
Soybean	15	15	15	15	8
Tomato M82-1-8-VF	16	17	17	17	16
Mean cfu cm^{-2} root	366	186	105	157	393

Range (cfu cm^{-2} root)

Kale	(593–1794)
Cabbage	(183–721)
Cowpea	(23–383)
Soybean	(9–186)
Tomato	(6–76)

[a] Plants are marked in order of their ability to support the fungus in the rhizosphere (1 = best) in each block; one replicate in each block.

TABLE 2

Abundance of *V. chlamydosporium* on the Roots of Bean Plants in Treated and Untreated Soil following a Kale Crop and its Effect on the Control of the Root Knot Nematodes *M. incognita*[a]

		Rhizosphere colonisation (cfu × 10^3 g^{-1} root) Kale[b]			*Females g^{-1} root Kale*			*Eggs × 10^3 per root system Kale*		
Crop		+	−	*Mean*	+	−	*Mean*	+	−	*Mean*
Bean	+	2·26	3·51	2·89	45	34	40	26·3	64·4	45·4
	−	2·18	0·00	1·10	39	42	41	59·4	174·2	116·8
	Mean	2·22	1·76		42	38		42·9	119·3	
	$SED_{interaction}$		1·02			NS			60·8	

[a] Means of four replicates.

[b] (+) soil treated with 5000 chlamydospores before crop planted or (−) no treatment.

root exudation[26] which enhanced fungal growth, or differences in the numbers of egg masses produced on the different plants influenced fungal abundance. When 1000 second-stage juveniles of *Meloidogyne incognita* (Kofoid & White) Chitwood were added around the roots of tomato plants growing in soil inoculated with 5000 chlamydospores of *V. chlamydosporium*, there was little increase in the growth of the fungus in the first five weeks after addition of nematodes (Fig. 5). However, after this time there was a significant increase in the abundance of the fungus on galled, but not on ungalled, roots from nematode-infected plants, and no increase on healthy plants. The increase in the density of the fungus occurred at the time at which egg masses were produced on the root surface.

Better control of root knot nematodes is usually achieved on roots where the fungus is abundant than where it is scarce. However, this simple relationship is affected by nematode density and plant susceptibility; large numbers of nematodes on susceptible plants produce large galls in which many egg masses remain embedded and escape attack by the fungus, which is confined to the rhizosphere. Also, similar numbers of colony forming units on the roots of oilseed rape and sugar beet did not result in the same amount of control of the beet cyst nematode, *Heterodera schachtii* Schmidt.[27] Fewer female nematodes survived on oilseed rape roots treated with *V. chlamydosporium*; differences in the distribution of the nematode and/or differences in the physiological state of the fungus on the two plant species may have affected the amount of control achieved.

5 *VERTICILLIUM CHLAMYDOSPORIUM* AS A BIOLOGICAL CONTROL AGENT

Verticillium chlamydosporium can be grown readily *in vitro* on a range of media but it has proved difficult to produce large numbers of chlamydospores in liquid fermenters, which are the favoured method for commercial

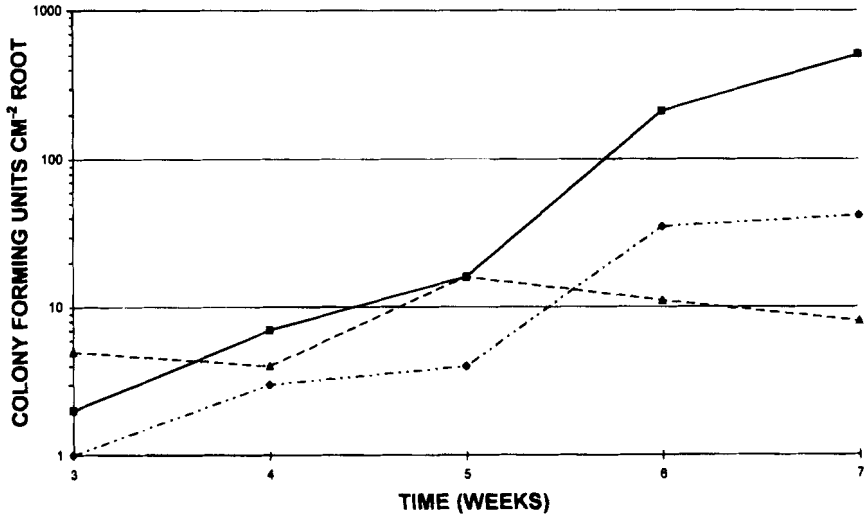

Fig. 5. Changes in the abundance of *Verticillium chlamydosporium* (cfu cm^{-2} root) (▲) on the surface of healthy uninfected tomato roots; (◆) on non-galled roots from tomato plants parasitised by *Meloidogyne incognita* and (■) on galled roots from the same infected plants. (Means of four replicates)

production.[28] For small-scale field trials sufficient inoculum can be produced on solid media[17] and waste products may be exploited. In experiments in the glasshouse[29] and microplots,[30] significant control of root knot nematodes has been achieved from applications of the fungus thoroughly mixed throughout the soil. However, it is impractical to incorporate the fungus in this way on a field scale and methods of restricting applications such as in-row treatments and bare root dips may be essential for commercial exploitation. Little work has been done on the methods of application of biological control agents for plant parasitic nematodes. Hence, initial use should be targeted towards root knot control on horticultural and ornamental crops, in private gardens or in subsistence farming systems, where there may be several opportunities to introduce the fungus during the crop cycle and where only small areas of soil would need treatment.

Verticillium chlamydosporium is not a potential replacement for nematicides and will not prevent large nematode infestations from causing significant yield losses in susceptible crops. However, at relatively low nematode infestations, and especially in association with poor hosts for the nematode, significant control may be achieved following applications of the fungus and a strategy has been devised for its practical exploitation.[18] On poor hosts such as kale and beans, few root-knot nematode juveniles invade the roots and galls are relatively small, so most egg masses are present in the rhizosphere and exposed to parasitism by *V. chlamydosporium.* Thus, the fungus enhances the impact of the poor host and increases the reduction in nematode infestations before the next susceptible crop in the rotation. This strategy is being tested in small plots in a plastic tunnel house in the UK for the control of *M. incognita* on vegetable crops. Soil infested with the nematode was treated with chlamydospores (5000 g^{-1}), applied around the roots of transplanted crops; the fungus was applied either to both crops in the cycle or only the first crop and control plants were untreated. Preliminary results are presented in Table 2 for a rotation in which an untreated tomato crop was followed by kale and then beans. In those soils treated with *V. chlamydosporium* before both crops, there was no significant increase in the amount of fungus on the roots compared to single applications before either beans or kale. Although similar numbers of females developed on the bean roots in all treatments, there was a significant reduction in the numbers of eggs produced on beans treated with the fungus, especially in soil treated twice, where final populations were 85% smaller than in untreated soils. However, many nematodes remained and it is not clear whether damage to succeeding crops would be reduced. It is difficult to work with root knot nematodes in the UK, even with protected crops, because the rates of nematode development and crop growth are poorer than in more typical warmer climates. To assess effectively the potential of *V. chlamydosporium* as a biological control agent, small plot trials are needed in more practical conditions.

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REFERENCES

1. Oostendorp, M., Dickson, D. W. & Mitchell, D. J., Population development of *Pasteuria penetrans* on *Meloidogyne arenaria. J. Nematol.*, **23** (1991) 58–64.
2. Gair, R., Mathias, P. L. & Harvey, P. N., Studies of cereal cyst-nematode populations and cereal yields under continuous or intensive culture. *Ann. Appl. Biol.*, **63** (1969) 503–12.
3. Kerry, B. R., Crump, D. H., & Mullen, L. A., Studies of the cereal cyst-nematode, *Heterodera avenae*, under continuous cereals, 1975–1978. II. Fungal parasitism of nematode females and eggs. *Ann. Appl. Biol.*, **100** (1982) 489–99.
4. Kerry, B. R., Biological control. In *Principles and Practice of Nematode Control in Crops*, ed. R. H. Brown & B. R. Kerry. Academic Press, Sydney, 1987, pp. 133–263.
5. Jones, F. G. W. & Kempton, R. A., Population dynamics, population models and integrated control. In *Plant Nematology*, ed. J. F. Southey. HMSO, London, 1978, pp. 333–61.
6. Ferris, H. & Noling, J. W., Analysis and prediction as a basis for management decisions. In *Principles and Practice of Nematode Control in Crops*, ed. R. A. Brown & B. R. Kerry. Academic Press, Sydney, 1987, pp. 49–85.
7. Stirling, G. R., *Biological Control of Plant Parasitic Nematodes.* CAB International, Wallingford, UK. (1991) pp. 1–282.
8. May, R. M. & Hassell, M. P., Population dynamics and biological control. *Phil. Trans. R. Soc. Lond. B318*, (1988) 129–69.
9. Jaffee, B. A., Population biology and biological control of nematodes. *Can. J. Microbiol.*, **38** (1992) 359–64.
10. Stirling, G. R. & Wachtel, M. F., Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica*, **26** (1980) 308–12.
11. Crump, D. H. & Kerry, B. R., A quantitative method for extracting resting spores of two nematode parasitic fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium*, from soil. *Nematologica*, **26** (1981) 330–9.
12. Eren, J. & Pramer, D. The most probable number of nematode-trapping fungi in soil. *Soil Sci.*, **99** (1965) 285.
13. Mitchell, D. J., Kannwischer-Mitchell, M. E. & Dickson, D. W. A semi selective medium for the isolation of *Paecilomyces lilacinus* from soil. *J. Nematol.*, **19** (1987) 255–6.
14. Gaspard, J. T., Jaffee, B. A. & Ferris, H., Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. *J. Nematol.*, **22** (1990) 207–13.
15. Kerry, B. R., Kirkwood, I. A., Leij, de F. A. A. M., Barba, J., Leijdens, M. B. & Brookes, P. C., Growth and survival

of *Verticillium chlamydosporium* Goddard, a parasite of nematodes, in soil. *Biocon. Sci. Tech.*, **3** (1993) 355–65.
16. Bourne, J. M., Kerry, B. R. & Leij, de F. A. A. M., Methods for the study of *Verticillium chlamydosporium* in the rhizosphere. *J. Nematol.*, **26** (1994) 587–91.
17. Leij, de F. A. A. M. & Kerry, B. R., The nematophagous fungus, *Verticillium chlamydosporium*, as a potential biological control agent for *Meloidogyne arenaria*. *Rev. Nematol.*, **14** (1991) 157–64.
18. Kerry, B. R., Ecological considerations for the use of the nematophagous fungus, *Verticillium chlamydosporium*, to control plant parasitic nematodes. *Can. J. Bot.* **73** (1995) S65–73.
19. Kerry, B. R., Two microorganisms for the biological control of plant parasitic nematodes. *Proc. Brighton Crop Prot. Conf.—Pests and Diseases* (1988). pp. 603–7.
20. Segers, R., Butt, T. M., Kerry, B. R. & Peberdy, J. F., The nematophagous fungus *Verticillium chlamydosporium* produces a chymoelastase-like protease that hydrolyses host nematode proteins *in situ*. *Microbiology*, **140** (1994) 2715–23.
21. Bonants, P. J. M., Fitters, P. F. L., Thijs, H., Belder, den E., Waalwijk, C. & Henfling, J. W. D. M., A basic serine protease from *Paecilomyces lilacinus* with biological activity against *Meloidogyne hapla* eggs. *Microbiology*, **141** (1995) 775–84.
22. Lopez-Llorca, L. V., Purification and properties of extracellular proteases produced by the nematophagous fungus *Verticillium suchlasporium*. *Can. J. Microbiol.*, **36** (1990) 530–7.
23. Tunlid, A., Rosén, S., Ek, B. & Rask, L., Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. *Microbiology*, **140** (1994) 1687–95.
24. Kerry, B. R. & Andersson, S., *Nematophthora gynophila* och *Verticillium chlamydosporium*, svampparasiter pa cystnematoder, vanliga i svenska jordar med förekomst ar strasadescystnematoder. *Vaxtskyddsnotiser*, **47** (1984) 5–6.
25. Leij, de F. A. A. M., Dennehy, J. A. & Kerry, B. R., Effect of watering on the distribution of *Verticillium chlamydosporium* in soil and the colonisation of egg masses of *Meloidogyne incognita* by the fungus. *Nematologica*, **39** (1993) 250–65.
26. Dorhout, R., Gommers, F. J. & Kollöffel, C., Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. *Physiol. Mol. Pl. Path.*, **43** (1993) 1–10.
27. Clyde, J., Studies on the infection of *Heterodera schachtii* by the nematophagous fungus *Verticillium chlamydosporium*. In *New approaches in biological control of soilborne diseases*, Vol. 15/1, ed. D. F. Jensen, J. Hockenhull & N. J. Fokkema. IOBC/WPRS Bulletin, 1992, 67–9.
28. Powell, K. A., The commercial exploitation of microorganism in agriculture. In: *Exploitation of Microorganisms*, ed. D. G. Jones. Chapman & Hall, London, 1993, pp. 441–59.
29. Godoy, G., Rodriguez-Kabana, R. & Morgan-Jones, G., Fungal parasites of *Meloidogyne arenaria* eggs in an Alabama soil. A mycological survey and greenhouse studies. *Nematropica*, **13** (1983) 201–13.
30. Leij, de F. A. A. M., Kerry, B. R. & Dennehy, J. A., *Verticillium chlamydosporium* as a biological control agent for *Meloidogyne incognita* and *M. hapla* in pot and microplot tests. *Nematologica*, **39** (1993) 115–26.